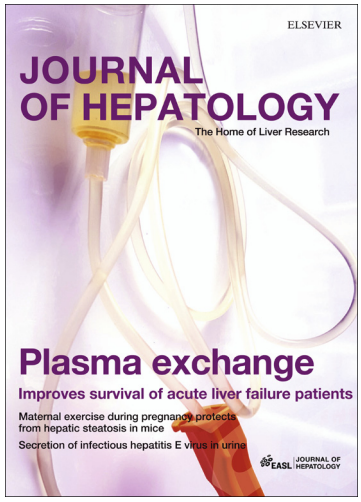
DOI:
10.1016/j.jhep.2016.03.010

Document Version
Peer reviewed version

Accepted Manuscript



Review

Update on alpha-1 antitrypsin deficiency: new therapies

David A. Lomas, John R. Hurst, Bibek Gooptu

PII: S0168-8278(16)30083-6
DOI: http://dx.doi.org/10.1016/j.jhep.2016.03.010
Reference: JHEPAT 6049

To appear in: *Journal of Hepatology*

Received Date: 30 December 2015
Revised Date: 16 March 2016
Accepted Date: 20 March 2016

Please cite this article as: Lomas, D.A., Hurst, J.R., Gooptu, B., Update on alpha-1 antitrypsin deficiency: new therapies, *Journal of Hepatology* (2016), doi: http://dx.doi.org/10.1016/j.jhep.2016.03.010

Update on alpha-1 antitrypsin deficiency: new therapies

David A. Lomas[1,2,3], John R. Hurst[1,2] and Bibek Gooptu[2,3,4]

[1]UCL Respiratory, Division of Medicine, Rayne Building, University College London, UK, [2]The London Alpha-1-Antitrypsin Deficiency Service, Royal Free London NHS Foundation Trust, London, UK, [3]Institute of Structural and Molecular Biology, UCL/Birkbeck College, University of London, London WC1E 7HX, UK, [4]Division of Asthma, Allergy and Lung Biology, King's College London, Guy's Hospital, 5th Floor, Tower Wing, London, UK.

Funding: DAL is supported by the Medical Research Council (UK) and the NIHR UCLH Biomedical Research Centre.

Author's contributions: DAL wrote the first draft of the manuscript that was revised by JRH and BG. All authors approved the final version.

Abstract

α_1-antitrypsin deficiency is characterised by the misfolding and intracellular polymerisation of mutant α_1-antitrypsin within the endoplasmic reticulum of hepatocytes. The retention of mutant protein causes hepatic damage and cirrhosis whilst the lack of an important circulating protease inhibitor predisposes the individuals with severe α_1-antitrypsin deficiency to early onset emphysema. Our work over the past 25 years has led to new paradigms for the liver and lung disease associated with α_1-antitrypsin deficiency. We review here the molecular pathology of the cirrhosis and emphysema associated with α_1-antitrypsin deficiency and show how an understanding of this condition provided the paradigm for a wider group of disorders that we have termed the serpinopathies. The detailed understanding of the pathobiology of α_1-antitrypsin deficiency has identified important disease mechanisms to target. As a result, several novel parallel and complementary therapeutic approaches are in development with some now in clinical trials. We provide an overview of these new therapies for the liver and lung disease associated with α_1-antitrypsin deficiency.

Keywords: serpins; cirrhosis; emphysema; therapeutic strategies; polymerisation

Introduction

We have described a group of protein conformational diseases that we have termed the serpinopathies [1]. They are characterised by the misfolding and intracellular polymerisation of members of the serine protease inhibitor or serpin superfamily. The best characterised of the serpinopathies is α_1-antitrypsin deficiency [2]. This is one of the most common genetic disorders with the severe Z deficiency allele (Glu342Lys) being present in 1:25 of the North European Caucasian population of whom 1:2000 are homozygotes. The Z mutation causes the retention of protein within hepatocytes in association with neonatal hepatitis, cirrhosis and hepatocellular carcinoma [3-5]. There is no specific treatment for the liver disease associated with α_1-antitrypsin deficiency which accounts for 3.5% and 1.1% of paediatric and adult liver transplants in the UK respectively.

The lack of α_1-antitrypsin, an important protease inhibitor, predisposes the Z homozygote to early onset panlobular basal emphysema [6]. α_1-antitrypsin deficiency is the only known genetic cause of emphysema and is found in 1-2% of all individuals with chronic obstructive pulmonary disease (COPD); COPD will be the third commonest cause of death worldwide by 2020. The only treatment directly targeting the underlying pathobiology of the lung disease is α_1-antitrypsin augmentation therapy which costs approximately $100,000/patient/year. Alpha$_1$-antitrypsin deficiency accounts for 3.2% of adult lung transplants and 10% of all lung transplants for emphysema in the UK. Our work over the past 25 years has led to a new paradigm for α_1-antitrypsin deficiency. Here we review the molecular basis of α_1-antitrypsin deficiency and provide an update of the therapeutic strategies that are being developed.

Polymerisation: the central feature of α_1-antitrypsin deficiency

Alpha$_1$-antitrypsin is the archetypal member of the serpin superfamily. The wild-type M protein is a 394 residue, 52kDa glycoprotein that is synthesised by hepatocytes, but is also produced by lung and gut epithelial cells,

neutrophils and alveolar macrophages. Alpha$_1$-antitrypsin is the major circulating antiprotease but its key function is regulation of the proteolytic effects of neutrophil elastase within the lung. We showed that the severe Z deficiency mutant of α_1-antitrypsin is retained within the endoplasmic reticulum (ER) of hepatocytes as ordered polymers that become sequestered in Periodic Acid Schiff-positive, diastase-resistant inclusions [2, 7]. We have used biophysical and crystallographic techniques to dissect the pathway of α_1-antitrypsin polymerisation [8-12]. Our work suggests that the Z mutation perturbs its local environment (breach region, Fig. 1A) to favour population of an unstable intermediate that we termed M* [8] in which β-sheet A opens [2, 8] and the upper part of helix F unwinds [9, 13, 14]. The patent β-sheet A can then accept insertion of the reactive site loop motif. Sequential insertion of the loop of one α_1-antitrypsin molecule into β-sheet A of a neighbour to form first a loop-sheet dimer, and then longer species linking more molecules, is the simplest model to explain the formation of elongated polymers [2, 8, 15] (Fig. 1B(i)). Indeed, polymerisation is blocked by peptides that mimic the reactive loop sequence and so compete for binding to the insertion site in β-sheet A [2, 16]. We subsequently showed that the same process explains the profound plasma deficiency and hepatic inclusions of 3 other mutants of α_1-antitrypsin: Siiyama (Ser53Phe) [17], Mmalton (ΔPhe52) [18] and King's (His334Asp) [7]. Polymerisation also underlies the deficiency of the mild S (Glu264Val), I (Arg39Cys), Queen's (Lys154Asn) and Baghdad (Ala336Pro) alleles of α_1-antitrypsin [12, 14, 19, 20] but the rate of polymer formation is much slower in keeping with the absence of liver disease and only mild plasma deficiency. In many cases the reduction in the thermal stability of the native fold of α_1-antitrypsin caused by mutations directly correlates with the polymerogenic tendency [11]. This implies that the mutants' conformational behaviour is qualitatively similar to that of wild-type α_1-antitrypsin, but that destabilised states become accessible at lower temperatures. However in some cases, disease mutations appear to cause polymerisation more by altering the balance of conformational behaviour (kinetic destabilisation) between native and intermediate states to favour population of the latter [21]. Importantly one such exception is the Z variant, that appears to cause a

relatively mild thermal destabilisation but is highly polymerogenic [11, 12, 16]. Understanding the polymerogenic behaviour of the most clinically-significant variant is important as it has relevance for the readouts that may be used in screening for therapeutic agents [16, 22]. Conversely, understanding the behaviour of rarer or milder variants in addition opens the way to precision medicine approaches, analogous to recent advances in cystic fibrosis [23, 24].

Controversies on the structure of the pathological polymer

Our original description of polymers of Z α_1-antitrypsin envisaged a linkage between the reactive centre loop and β-sheet A [2] (Fig. 1B(i)). Clinically, Z α_1-antitrypsin inclusions within hepatocytes are increased by pyrexia and we assumed that polymers generated *in vitro* by heating purified Z α_1-antitrypsin would be identical to those formed *in vivo*. We further assumed that polymers generated *in vitro* would be identical whether they were formed by heating purified Z α_1-antitrypsin or incubation with denaturants (urea or guanidine) [8]. However we now know the latter assumption was incorrect, and the former assumption is also a matter of debate [22]. An alternative linkage was suggested by the crystal structures of a dimer of antithrombin in which the molecules were linked by a β-hairpin of the reactive centre loop and strand 5A [25] (Fig. 1B (ii)). The biophysical characteristics of polymers of α_1-antitrypsin formed by refolding from guanidine gave support to the β-hairpin linkage [26]. The cause of the disparate findings became clear with our development of the 2C1 monoclonal antibody that recognises polymers from the livers of individuals with α_1-antitrypsin deficiency [7]. This antibody binds polymers formed by heating monomeric α_1-antitrypsin, but not those formed by refolding from guanidine and urea [15]. Our NMR studies followed the polymerisation of Queen's (Lys154Asn) α_1-antitrypsin under physiological conditions or in urea. Intermediate (M*) formation under physiological conditions was associated with highly native-like behaviour with changes in a few key motifs [14]. Global changes were observed in urea consistent with more widespread unfolding, in keeping with data from hydrogen-deuterium exchange [27]. Consequently different polymeric linkages can be accessed by different

denaturing conditions. The application of heat to monomeric α_1-antitrypsin recapitulated the 2C1 neo-epitope of polymers associated with disease [15]. This neo-epitope is also observed in self-terminating trimers of Z α_1-antitrypsin artificially constrained by disulphide bonds and purified from the cytosol of a yeast expression system [28]. This species was crystallised and the structure determined to 3.9 Å resolution. The structure revealed yet another stable linkage mechanism, this time via complementary intermolecular insertion of the C-terminal triple-strand motif (Fig. 1B (iii)). This mechanism again requires a substantially unfolded intermediate state, associated with intramolecular insertion of the reactive site loop. Recent work using small-angle X-ray scattering (SAXS) suggested that the trimer, tetramer, and pentamer of Z α_1-antitrypsin all form ring-like structures in keeping with C-terminal domain-swap mechanism of polymerisation [29]. However ring structures are only rarely seen in inclusions from the livers of individuals with Z α_1-antitrypsin deficiency ([2] and Fig. 2).

Intracellular processing of α_1-antitrypsin polymers

Approximately 70% of Z α_1-antitrypsin is degraded within hepatocytes by ER associated degradation (ERAD) [30]. Fifteen percent folds effectively and is secreted, while the remainder self-associates to form polymers. These are in part degraded by autophagy, but a proportion persist in intractable inclusions [30, 31]. Misfolded proteins within the ER lumen usually trigger adaptive measures termed the unfolded protein response (UPR). Indeed, terminally misfolded truncated variants of α_1-antitrypsin (e.g., α_1-antitrypsin Null-HongKong and Saar) constitutively activate the UPR [32, 33]. Surprisingly, the accumulation of Z α_1-antitrypsin polymers within the ER is not associated with UPR activation in cell lines that recapitulate hepatocyte phenotypes or in transgenic mice, possibly because polymers are structurally ordered (Fig. 3). Instead Z α_1-antitrypsin expression activates NFκB by a calcium-mediated pathway that is independent of the UPR [32-35]. We have termed this the 'ordered protein response' [35] and others have shown that it results in the release of IL-6 and IL-8 [34]. These cytokines are well-recognised mediators of both acute and chronic inflammatory responses and so may play

pathogenic roles in both the liver and lung disease associated with α_1-antitrypsin deficiency. Moreover, cells expressing Z α_1-antitrypsin display a more marked UPR when stressed with a 'second hit' [33, 34]. We have proposed that this results from α_1-antitrypsin polymers increasing the viscosity within the ER thereby reducing the mobility of chaperones and hence their ability to neutralise the effect of a second insult [33].

Alpha$_1$-antitrypsin polymers, inflammation and emphysema

The study of the rare null mutations of α_1-antitrypsin provides compelling evidence that the lack of circulating α_1-antitrypsin plays a central role in the development of emphysema [36]. Consistent with this, α_1-antitrypsin variants that fold more successfully and are less polymerogenic than the Z variant have higher circulating levels of α_1-antitrypsin and appear to be associated with far lower risks of lung disease. Indeed such variants are typically described in association with lung disease in compound heterozygous states together with one Z allele (e.g. SZ individuals [37, 38]). The recognition that polymers were present within hepatocytes raised the question that they may also be present within the lung. Polymers are detectable in bronchoalveolar lavage from Z α_1-antitrypsin homozygotes [39]. They have even been found after a Z α_1-antitrypsin individual had undergone a liver transplant i.e. the subject received a M α_1-antitrypsin liver [40]. This elegant clinical experiment demonstrated that pulmonary polymers may arise from local production. However we have been unable to detect polymers in our studies assessing primary bronchial epithelial cells [41]. Polymers of α_1-antitrypsin form within the lung as a result of local inflammation and exposure to cigarette smoke [39, 40, 42, 43]. They are pro-inflammatory for human neutrophils *in vitro* [40, 44] and in murine lungs *in vivo* [42]. Moreover cigarette smoke induces both intrapulmonary polymer formation and neutrophil influx in transgenic mice that express Z, but not M α_1-antitrypsin [43]. Polymers have also been identified in the skin of an individual with α_1-antitrypsin deficiency and panniculitis and in a renal biopsy of an individual with α_1-antitrypsin deficiency and vasculitis. We have recently shown that circulating polymers of α_1-antitrypsin are present in

all individuals with ZZ α_1-antitrypsin deficiency (mean 36.3+/-SD33.3μg/mL; n=517) and that the level is associated with COPD (OR 3.6, 95% CI 1.4–9.1) [45]. These observations provide a new paradigm for the pathogenesis of the emphysema associated with α_1-antitrypsin deficiency - a combination of loss of anti-protease function combined with pro-inflammatory intrapulmonary polymers [44]. However it is still unknown whether the pro-inflammatory properties of polymers play an important role in the pathogenesis of the emphysema associated with α_1-antitrypsin deficiency or whether the majority of the pathology is driven by the deficiency in the antiproteinase screen (Fig. 3). Also, the balance of polymers derived from plasma Z α_1-antitrypsin and from local production within the lung is unresolved.

Polymers and the serpinopathies

Alpha$_1$-antitrypsin is a member of a family of serine proteinase inhibitors termed the serpins. We and others reported the same process of polymerisation in different members of the family in association with a range of diseases: polymerisation of mutants of C1-inhibitor are associated with plasma deficiency and angio-oedema; polymerisation of mutants of antithrombin cause plasma deficiency in association with thrombosis and the polymerisation of mutants of α_1-antichymotrypsin cause plasma deficiency that in some studies is associated with emphysema. A mutation in heparin co-factor II (Glu428Lys) that is homologous to the Z allele of α_1-antitrypsin is associated with plasma deficiency but as yet this has not been shown to cause disease [1, 46].

The process of disease-related polymerisation is most striking for mutations in the neurone-specific serpin neuroserpin. We showed that mutations in neuroserpin result in the formation of polymers that are retained as neuronal inclusions in the cerebral cortex [47]. These inclusions result in an autosomal dominant dementia that we called familial encephalopathy with neuroserpin inclusion bodies or FENIB [47, 48]. Six mutations have now been described that underlie FENIB [48-50]. We have assessed a range of mutants of different severity in purified recombinant protein, cell, fly, worm and mouse

models of disease [48, 51-57]. The data show a direct relationship between the severity of the mutation, the rate of polymer formation and the severity of the associated dementia. In view of the common molecular mechanism underlying these disorders we grouped them together as a new class of disease-the serpinopathies [1]. The serpinopathies can cause disease by (i) a toxic gain of function from the accumulated protein, as in α_1-antitrypsin deficiency [2] and the dementia FENIB [47] and (ii) from the lack of an important circulating inhibitor as occurs in deficiency of α_1-antitrypsin (emphysema), antithrombin (thrombosis), C1-inhibitor (angio-oedema) and α_1-antichymotrypsin (emphysema) [58, 59]. Moreover what is learned from α_1-antitrypsin and neuroserpin is likely to be applicable to an entire family of disease.

Towards an effective therapy for α_1-antitrypsin deficiency.

Behavioural approaches

The most effective current therapies for α_1-antitrypsin deficiency are preventive. It is essential that individuals with severe α_1-antitrypsin deficiency abstain from smoking, and ideally avoid passive exposures including dusty occupations [60]. This will help to preserve lung function. We also strongly advise against excessive consumption of alcohol §and avoiding excessive weight gain. Both alcohol consumption and high body-mass index (via fatty liver) can cause liver damage in addition to that caused by α_1-antitrypsin deficiency.

Treatment for lung disease associated with α_1-antitrypsin deficiency

(i) Replacement therapy

The mainstay of treatment for severe α_1-antitrypsin deficiency in many countries is the correction of the plasma deficiency with intravenous augmentation of pooled plasma purified α_1-antitrypsin [61]. Augmentation therapy is associated with a reduction in the frequency of respiratory tract infections and a decline in sputum markers of inflammation. Two randomised clinical trials have been undertaken but neither has shown that augmentation

therapy reduces the rate of decline in lung function [62, 63]. We have postulated that this is because the lung disease associated with α_1-antitrypsin deficiency is caused by more than the deficiency of an important antiprotease; the pro-inflammatory intrapulmonary polymers may negate the antiprotease effect of intravenous replacement therapy [44, 64]. Moreover, the relevance of decline in forced expiratory volume in 1 s (FEV_1) as an endpoint in respiratory disease is increasingly debated given recent data highlighting a developmental cause for reduced lung function [65]. The RAPID investigators have recently reported a 24 month study in which 93 individuals were randomised to active therapy and 87 to received placebo [66]. The annual rate of lung density loss at total lung capacity (TLC) and functional residual capacity (FRC) combined did not differ between groups. However, the annual rate of lung density loss at TLC alone was significantly less in patients in the group that received augmentation therapy (difference 0·74 g/L per year [95% confidence interval 0·06–1·42], p=0·03) but there was no difference at FRC alone (difference 0·48 g/L per year [–0·22 to 1·18], p=0·18). It is unclear how this difference at TLC translates to clinical benefit or if a subgroup can be identified that particularly benefit from augmentation therapy. Nevertheless the study has been interpreted positively by the European Medicines Agency who have recently granted a licence for augmentation therapy in individuals with α_1-antitrypsin deficiency. The UK National Institute for Clinical Excellence (NICE) will now need to rule whether the difference in lung density represents value for money in a managed health care system.

Augmentation therapy is not given with the intention of affecting the liver disease associated with α_1-antitrypsin deficiency which appears to be predominantly driven by misfolding and polymerisation rather than loss-of-function. Theoretically there may be benefit if the excessive synthesis, misfolding and polymerisation of Z α_1-antitrypsin is driven by an, as yet undefined, negative feedback loop driven by low levels of extracellular protein. Moreover, anti-apoptotic and anti-inflammatory effects of extracellular α_1-antitrypsin have been invoked to explain beneficial effects of α_1-antitrypsin in a mouse model of acute liver failure [67]. Nevertheless, to date no data have indicated any signal of benefit with this treatment in liver disease.

(ii) Alternative approaches to deliver α_1-antitrypsin replacement therapy

There are several alternative approaches to restore circulating levels of α_1-antitrypsin and so protect individuals against the development of emphysema. These include the production of recombinant α_1-antitrypsin for replacement therapy and the delivery of α_1-antitrypsin by an inhaled route that should significantly reduce the amount that is required to counter the effect of neutrophil elastase. A third approach is gene-based therapy using non-viral gene transfer, gamma-retrovirus, recombinant adenovirus (rAd), and recombinant adeno-associated virus (rAAV) vectors. One rAAV vector has been assessed in a phase II clinical trial, showing promise but only achieving 3-5% of the target range of α_1-antitrypsin [68].

(iii) Retinoic acid

Retinoic acid abrogates elastase-induced emphysema in rats [69] and so was evaluated in man [70]. 133 and 129 individuals with severe α_1-antitrypsin deficiency were randomised to placebo or 5 mg/day palovarotene (an oral γ-selective retinoid agonist) respectively for 1 year (the REPAIR study: Retinoid treatment of Emphysema in Patients on the α_1-antitrypsin International Registry). Palovarotene was generally well tolerated but there was no significant benefit on lung density in individuals with moderate-to-severe emphysema.

(iv) Symptomatic therapy, transplantation, lung volume reduction surgery and endobronchial valves

In the absence of specific therapy, individuals with α_1-antitrypsin deficiency lung disease should be considered for therapies used in α_1-antitrypsin replete COPD. These include pulmonary rehabilitation, vaccination and the use of inhaled pharmacotherapies including (alone or in combination) inhaled corticosteroids, long-acting beta-agonists and long-acting anti-cholinergics currently based on multi-component patient assessment of symptoms, lung function impairment and exacerbation frequency. It should be noted, however, that α_1-antitrypsin deficiency has often been an exclusion criteria in clinical trials of these approaches in 'usual' COPD.

The most effective therapy for individuals with severe airflow obstruction is single lung transplantation. However given the limited availability of donor organs, lung volume reduction surgery (LVRS) must be considered in these individuals possibly as definitive therapy, but more likely as a bridge to transplantation. However the benefits derived from LVRS in individuals with α_1-antitrypsin deficiency is inferior to that of individuals who have 'usual' COPD. Further work is necessary to determine whether there is a subgroup of individuals with α_1-antitrypsin deficiency who derive more long-lasting improvement from this intervention [71].

Endobronchial lung volume reduction (ELVR) performed by one-way valves inserted via a flexible bronchoscope can result in a moderate but significant improvement in lung function and exercise tolerance, eliminating the surgical risks. However most studies of this method have excluded individuals with α_1-antitrypsin deficiency. A case series reported the effect of treating 15 individuals with α_1-antitrypsin deficiency with one-way endobronchial valves. The recipients had gas trapping (residual volume of 140% or more), airflow obstruction (forced expiratory volume in 1 s (FEV_1) 15-45% of predicted) and severe heterogeneous emphysema. One individual coughed up valves after 2 months, 1 developed a pneumothorax and had valve displacement and subsequent removal and 1 improved from an FEV_1 of 0.62 to 0.84 liters, but after 4 months developed repeated and severe pneumonia and the valves had to be removed. The remaining 12 individuals were followed up for at least 1 year: FEV_1 increased by a mean of 54%, quality of life was much improved and 2 individuals were taken off oxygen therapy. There was no significant deterioration in lung function during the 4-year follow-up [72].

Treatment for liver disease associated with α_1-antitrypsin deficiency

Currently there is no disease-specific therapy for the hepatic manifestations of α_1-antitrypsin deficiency. However the detailed understanding of its pathobiology has both identified important disease mechanisms to target, and also made it an ideal model for the application of novel biomedical technologies. As a result, several novel parallel and complementary therapeutic approaches are in trials and in development.

(i) Increasing the clearance of inclusions by stimulating autophagy.

Different groups have assessed a range of chemical chaperones that stabilise intermediates on the Z α_1-antitrypsin folding pathway [73]. Some were effective in cell and animal models of disease [74] but human trials have been disappointing [75]. David Perlmutter's group (Pittsburgh, USA) have taken the exciting approach of using an FDA-approved drug, carbamazepine to stimulate degradation pathways to clear intracellular polymers of α_1-antitrypsin [76]. This is now being assessed in a clinical trial (NCT01379469). Autophagy may also be enhanced by rapamycin (sirolimus) when given in weekly dose pulses, rather than as a daily regimen. Weekly dosing of rapamycin increased the number of autophagic vacuoles and reduced the accumulation of intrahepatic polymerised Z α_1-antitrypsin. It also reduced other markers of hepatocellular injury including cleavage of caspase 12 and hepatic fibrosis [77]. Caspase 12 is one of the inflammatory caspases that has homology to caspases 4 and 5 in humans. It plays a role in ER stress induced apoptosis in the mouse but not in man [78]. Autophagy can be induced by viral vectors that overexpress the autophagy regulator transcription factor EB (TFEB). This reduced the accumulation of Z α_1-antitrypsin polymer, apoptosis and fibrosis in the liver of the transgenic mouse that expresses Z α_1-antitrypsin [79]. These approaches reduce intracellular α_1-antitrypsin burden and decrease hepatic fibrosis in a mouse model of disease. The challenge will be to demonstrate efficacy in man.

(ii) Modifying proteostasis networks.

An alternative approach is to consider chaperone-mediated folding and degradation as part of a robust and cooperative proteostatic network. Suberoylanilide hydroxamic acid (SAHA) increases the secretion of Z α_1-antitrypsin from epithelial cell lines to a level of 50% of that observed for wild-type α_1-antitrypsin [80]. It is believed to act in part by inhibition of histone deacetylase (HDAC)7. Such strategies also have the potential to boost secretion of less polymerogenic α_1-antitrypsin variants to ameliorate lung pathology e.g. in SZ compound heterozygotes. However this approach has yet to be assessed in animal models of disease.

(iii) Silencing expression of Z α$_1$-antitrypsin.

Two pharmaceutical companies (Arrowhead Research Corporation and Alnylam Pharmaceuticals Inc.) have developed RNA interference (RNAi)-based approaches to silence Z α$_1$-antitrypsin production in hepatocytes [81, 82]. These use small interfering (si)RNA constructs targeted against mRNA encoding human α$_1$-antitrypsin and conjugated with hepatocyte targeting motifs. Treatment reduces circulating levels of α$_1$-antitrypsin and both soluble and aggregated hepatic α$_1$-antitrypsin protein in transgenic mice expressing Z α$_1$-antitrypsin [83]. Moreover administration of the siRNA constructs arrests the progression of liver disease in these animals after short-term treatment, reverses liver disease after long-term treatment, decreases liver fibrosis and prevents liver disease in young animals. It also leads to marked reduction in liver fibrosis. The administration of RNAi treatment to non-human primates led to an approximately 80% reduction in levels of circulating normal α$_1$-antitrypsin, demonstrating potential for this approach in man. RNAi thus represents a promising therapy for α$_1$-antitrypsin deficiency related liver disease and is now in a phase I/II clinical trial (NCT02503683). The study started in July 2016 and aims to assess safety, tolerability, pharmacokinetics and pharmacodynamics of subcutaneously administered antisense α$_1$-antitrypsin in healthy adult subjects and individuals with Z α$_1$-antitrypsin deficiency liver disease.

(iv) Small molecule approach to block intracellular polymerisation.

We have used our understanding that Z α$_1$-antitrypsin polymerisation underlies the liver accumulation and subsequent plasma deficiency to develop novel strategies to attenuate polymerisation and thereby 'cure' α$_1$-antitrypsin deficiency. Our early work showed that reactive loop peptides could bind to α$_1$-antitrypsin and block polymerisation of purified protein *in vitro* [2, 84, 85]. Smaller, refined peptides were developed that had a similar effect with more specificity for Z, rather than M, α$_1$-antitrypsin [16, 86-88]. However the challenge is to deliver these peptides to cells, let alone to a human liver. A more recent approach has been to use our understanding of the structural biology of polymers to develop small molecules to block polymerisation. The

starting point was the identification of a hydrophobic pocket in α_1-antitrypsin that is bounded by strand 2A and helices D and E [89]; Fig. 4. The cavity is patent in the native protein but is filled as β-sheet A accepts an exogenous reactive loop peptide during polymerisation. The introduction of Thr114Phe on strand 2 of β-sheet A filled this cavity, retarded polymer formation *in vitro* and increased the secretion of Z α_1-antitrypsin from a *Xenopus* oocyte expression system [46, 90]. This cavity was used as a target for rational structure-based drug design to block polymer formation [91]. Virtual ligand screening was performed on 1.2 million small molecules and 66 compounds were identified as potential binders. Compounds were identified that completely blocked polymerisation *in vitro* and in cell models of diseas [91]. This work represented a 'proof of principle' rather than a new therapy. It was the basis of a DPAc (Discovery Partnership with Academia) partnership with GlaxoSmithKline to develop small molecules that are effective in treating α_1-antitrypsin deficiency (*http://www.cam.ac.uk/research/news/new-collaboration-to-develop-treatments-for-liver-disease/*).

(v) *Intrabodies to block intracellular polymerisation and increase secretion*

More recently we have used monoclonal antibody technology to identify interactors with Z α_1-antitrypsin. This has proved to be powerful technology that has allowed us to identify antibodies that specifically detect the polymeric [7] and latent [92] conformers of α_1-antitrypsin and antibodies that can accelerate [93] and block [94] polymer formation. The 4B12 monoclonal antibody blocked α_1-antitrypsin polymerisation *in vitro* at a 1:1 molar ratio, causing a small increase of the stoichiometry of inhibition of neutrophil elastase [94]. Expression of a single chain-variable-fragment (scFv) intrabody of mAb4B12 within the ER reduced the intracellular polymerisation of Z α_1-antitrypsin by 60% and increased the secretion of Z α_1-antitrypsin that retained inhibitory activity against neutrophil elastase. The 4B12 monoclonal antibody recognises a discontinuous epitope in E32/H43/L306 α_1-antitrypsin, and acts by altering protein dynamics rather than binding preferentially to the native state, analogous to the Z mutation. This novel approach could reveal new target sites for small-molecule intervention that block the transition to

aberrant polymers without compromising the inhibitory activity of Z α_1-antitrypsin [94].

(vi) Stem cell technology to generate models of disease and novel therapies for α_1-antitrypsin deficiency

Human induced pluripotent stem cells (hIPSCs) promise great opportunities for modelling of human disease as they allow the expression of proteins of interest from endogenous promoters. Dermal fibroblasts have been isolated from individuals with α_1-antitrypsin deficiency and used to generate patient-specific hIPSC lines. Each of the hIPSC lines was differentiated into 'hepatocyte-like cells' using a novel and simple three step differentiation protocol in chemically-defined conditions. The patient-specific hIPSC derived hepatocytes show the key pathological features of α_1-antitrypsin deficiency: protein misfolding, the formation of pathological polymers and the retention of polymers in the ER [95, 96]. These cells provide an invaluable resource for modelling α_1-antitrypsin deficiency and genetic diseases. We extended this work to correct the genetic defect responsible for Z α_1-antitrypsin deficiency within the hIPSC. This was targeted using a combination of engineered Zinc finger nucleases and a piggyBac donor vector [97]. This efficient gene editing technique restored normal structure, function and secretion of α_1-antitrypsin in subsequently derived liver cells. These cells secreted both albumin and α_1-antitrypsin when introduced into a mouse model of liver injury. The challenge is to refine the strategy, most likely with CRISPR technology, to obtain cells that are more like the fully-differentiated hepatocyte and which are safe to use in man. Nevertheless such an approach may address both the liver and lung disease associated with α_1-antitrypsin deficiency.

A Clinical Service Perspective

We believe that patients with α_1-antitrypsin deficiency are best cared for in collaboration with centres that offer joint respiratory-hepatology expertise. Such services need access to appropriate diagnostics (including sequencing for the SERPINA1 gene that encodes α_1-antitrypsin) and clinical genetics. We have therefore established these within the London Alpha-1 Antitrypsin

Deficiency Service. Here we monitor the lung manifestations with regular spirometry, lung volumes and gas transfer supplemented by CT scan where this would alter management. We monitor the liver manifestations using liver biochemistry and AFP, transient elastography and liver ultrasound. We have a low threshold of liver biopsy when the above tests are abnormal. The management of lung disease in the UK is as for α_1-antitrypsin replete COPD. From a liver perspective, patients with α_1-antitrypsin deficiency are advised to maintain a normal body composition and not drink alcohol to excess. As outlined here, further research is likely to lead to clear-cut benefits for the clinical management of α_1-antitrypsin deficiency in the relatively near future. Translational research capacity is therefore integrated into the service, including the opportunity for patients to participate in new therapeutic trials as they arise.

Acknowledgments

DAL is supported by the Medical Research Council (UK) and the NIHR UCLH Biomedical Research Centre.

References

[1] Lomas DA, Mahadeva R. Alpha-1-antitrypsin polymerisation and the serpinopathies: pathobiology and prospects for therapy. J Clin Invest 2002;110:1585-1590.
[2] Lomas DA, Evans DL, Finch JT, Carrell RW. The mechanism of Z α_1-antitrypsin accumulation in the liver. Nature 1992;357:605-607.
[3] Sveger T. Liver disease in alpha$_1$-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med 1976;294:1316-1321.
[4] Eriksson S, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha$_1$-antitrypsin deficiency. N Engl J Med 1986;314:736-739.
[5] Dawwas MF, Davies SE, Griffiths WJ, Lomas DA, Alexander GJ. Prevalence and risk factors for liver involvement in individuals with PiZZ-related lung disease. Am J Respir Crit Care Med 2013;187:502-508.
[6] Eriksson S. Studies in α_1-antitrypsin deficiency. Acta Med Scand 1965;suppl.432:1-85.
[7] Miranda E, Pérez J, Ekeowa UI, Hadzic N, Kalsheker N, Gooptu B, et al. A novel monoclonal antibody to characterise pathogenic polymers in liver disease associated with α_1-antitrypsin deficiency. Hepatology 2010;52:1078-1088.
[8] Dafforn TR, Mahadeva R, Elliott PR, Sivasothy P, Lomas DA. A kinetic mechanism for the polymerisation of α_1-antitrypsin. J Biol Chem 1999;274:9548-9555.
[9] Gooptu B, Hazes B, Chang W-SW, Dafforn TR, Carrell RW, Read R, et al. Inactive conformation of the serpin α_1-antichymotrypsin indicates two stage insertion of the reactive loop; implications for inhibitory function and conformational disease. Proc Natl Acad Sci (USA) 2000;97:67-72.
[10] Haq I, Irving JA, Faull SV, Dickens JA, Ordóñez A, Belorgey D, et al. Reactive centre loop mutants of α-1-antitrypsin reveal position-specific effects on intermediate formation along the polymerization pathway. Biosci Rep 2013;33:e00046.
[11] Irving JA, Haq I, Dickens JA, Faull SV, Lomas DA. Altered native stability is the dominant basis for susceptibility of α1-antitrypsin mutants to polymerization. Biochem J 2014;460:103-115.
[12] Haq I, Irving JA, Saleh AD, Dron L, Regan-Mochrie GL, Motamedi-Shad N, et al. Deficiency mutations of α1-antitrypsin differentially affect folding, function and polymerization. Am J Resp Cell Mol Biol 2015;In press.
[13] Gooptu B, Miranda E, Nobeli I, Mallya M, Purkiss A, Leigh Brown SC, et al. Crystallographic and cellular characterisation of two mechanisms stabilising the native fold of alpha-1-antitrypsin: implications for disease and drug design. J Mol Biol 2009;387:857-868.
[14] Nyon MP, Segu L, Cabrita LD, Lévy GR, Kirkpatrick J, Roussel BD, et al. Structural dynamics associated with intermediate formation in an archetypal conformational disease. Structure 2012;20:504-512.
[15] Ekeowa UI, Freekeb J, Miranda E, Gooptu B, Bush MF, Pérez J, et al. Defining the mechanism of polymerization in the serpinopathies. Proc Natl Acad Sci USA 2010;107:17146-17151.
[16] Nyon MP, Prentice T, Day J, Kirkpatrick J, Sivalingam GN, Levy G, et al. An integrative approach combining ion mobility mass spectrometry, X-ray

crystallography and NMR spectroscopy to study the conformational dynamics of α1-antitrypsin upon ligand binding. Protein Sci 2015;24:1301-1312.

[17] Lomas DA, Finch JT, Seyama K, Nukiwa T, Carrell RW. α_1-antitrypsin S_{Iiyama} (Ser^{53}→Phe); further evidence for intracellular loop-sheet polymerisation. J Biol Chem 1993;268:15333-15335.

[18] Lomas DA, Elliott PR, Sidhar SK, Foreman RC, Finch JT, Cox DW, et al. $Alpha_1$-antitrypsin Mmalton (^{52}Phe deleted) forms loop-sheet polymers *in vivo*: evidence for the C sheet mechanism of polymerisation. J Biol Chem 1995;270:16864-16870.

[19] Elliott PR, Stein PE, Bilton D, Carrell RW, Lomas DA. Structural explanation for the dysfunction of S α_1-antitrypsin. Nat Struct Biol 1996;3:910-911.

[20] Mahadeva R, Chang W-SW, Dafforn TR, Oakley DJ, Foreman RC, Calvin J, et al. Heteropolymerisation of S, I and Z α_1-antitrypsin and liver cirrhosis. J Clin Invest 1999;103:999-1006.

[21] Knaupp AS, Levina V, Robertson AL, Pearce MC, Bottomley SP. Kinetic instability of the serpin Z α_1-antitrypsin promotes aggregation. J Mol Biol 2010;396:375-383.

[22] Nyon MP, Gooptu B. Therapeutic targeting of misfolding and conformational change in α1-antitrypsin deficiency. Future Med Chem 2014;6:1047-1065.

[23] Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, et al. Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc Natl Acad Sci U S A 2009;106:18825-18830.

[24] Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, et al. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. Proc Natl Acad Sci U S A 2011;108:18843-18848.

[25] Yamasaki M, Li W, Johnson DJ, Huntington JA. Crystal structure of a stable dimer reveals the molecular basis of serpin polymerization. Nature 2008;455:1255-1258.

[26] Krishnan B, Gierasch LM. Dynamic local unfolding in the serpin α-1 antitrypsin provides a mechanism for loop insertion and polymerization. Nat Struct Mol Biol 2011;18:222-226.

[27] Tsutsui Y, Dela Cruz R, Wintrode PL. Folding mechanism of the metastable serpin α1-antitrypsin. Proc Natl Acad Sci U S A 2012;109:4467-4472.

[28] Yamasaki M, Sendall TJ, Pearce MC, Whisstock JC, Huntington JA. Molecular basis of α1-antitrypsin deficiency revealed by the structure of a domain-swapped trimer. EMBO Rep 2011;12:1011-1017.

[29] Behrens MA, Sendall TJ, Pedersen JS, Kjeldgaard M, Huntington JA, Jensen JK. The shapes of Z-a1-antitrypsin polymers in solution support the C-terminal domain-swap mechanism of polymerization. Biophysical J 2014;107:1905-1912.

[30] Kröger H, Miranda E, MacLeod I, Pérez J, Crowther DC, Marciniak SJ, et al. Endoplasmic reticulum-associated degradation (ERAD) and autophagy cooperate to degrade polymerogenic mutant serpins. J Biol Chem 2009;284:22793-22802.

[31] Teckman JH, Perlmutter DH. Retention of mutant α_1-antitrypsin Z in endoplasmic reticulum is associated with an autophagic response. Am J Physiol Gastrointest Liver Physiol 2000;279:G961-G974.
[32] Hidvegi T, Mirnics K, Hale P, Ewing M, Beckett C, Perlmutter DH. Regulator of G signaling 16 is a marker for the distinct ER stress state associated with aggregated mutant alpha 1-antitrypsin Z in the classical form of a_1-antitrypsin deficiency. J Biol Chem 2007;282:27769-27780.
[33] Ordóñez A, Snapp EL, Tan L, Miranda E, Marciniak SJ, Lomas DA. Endoplasmic reticulum polymers impair luminal protein mobility and sensitize to cellular stress in alpha-1-antitrypsin deficiency. Hepatology 2013;57:2049-2060.
[34] Lawless MW, Greene CM, Mulgrew A, Taggert CC, O'Neill SJ, McElvaney NG. Activation of endoplasmic reticulum-specific stress responses associated with the conformational disease Z α_1-antitrypsin deficiency. J Immunol 2004;172:5722-5726.
[35] Davies MJ, Miranda E, Roussel BD, Kaufman RJ, Marciniak SJ, Lomas DA. Neuroserpin polymers activate NF-kB by a calcium signalling pathway that is independent of the unfolded protein response. J Biol Chem 2009;284:18202-18209.
[36] Fregonese L, Stolk J, Frants RR, Veldhuisen B. Alpha-1 antitrypsin Null mutations and severity of emphysema. Respir Med 2008;102:876-884.
[37] Turino GM, Barker AF, Brantly ML, Cohen AB, Connelly RP, Crystal RG, et al. Clinical features of individuals with PI*SZ phenotype of α_1-antitrypsin deficiency. Am J Respir Crit Care Med 1996;154:1718-1725.
[38] Seersholm N, Kok-Jensen A. Intermediate alpha 1-antitrypsin deficiency PiSZ: a risk factor for pulmonary emphysema? Respir Med 1998;92:241-245.
[39] Elliott PR, Bilton D, Lomas DA. Lung polymers in Z α_1-antitrypsin related emphysema. Am J Respir Cell Mol Biol 1998;18:670-674.
[40] Mulgrew AT, Taggart CC, Lawless MW, Greene CM, Brantly ML, O'Neill SJ, et al. Z α_1-antitrypsin polymerizes in the lung and acts as a neutrophil chemoattractant. Chest 2004;125:1952-1957.
[41] van 't Wout EF, Dickens JA, van Schadewijk A, Haq I, Kwok HF, Ordóñez A, et al. Increased ERK signalling promotes inflammatory signalling in primary airway epithelial cells expressing Z α1-antitrypsin. Hum Mol Genet 2014;23:929-941.
[42] Mahadeva R, Atkinson C, Li J, Stewart S, Janciauskiene S, Kelley DG, et al. Polymers of Z α_1-antitrypsin co-localise with neutrophils in emphysematous alveoli and are chemotactic *in vivo*. Am J Pathol 2005;166:377-386.
[43] Alam S, Li Z, Janciauskiene S, Mahadeva R. Oxidation of Z alpha-1-antitrypsin by cigarette smoke induces polymerization: a novel mechanism of early-onset emphysema. Am J Respir Cell Mol Biol 2011;45:261-269.
[44] Parmar JS, Mahadeva R, Reed BJ, Farahi N, Cadwallader K, Bilton D, et al. Polymers of α_1-antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema. Am J Respir Cell Mol Biol 2002;26:723-730.
[45] Tan L, Dickens JA, Demeo DL, Miranda E, Perez J, Rashid ST, et al. Circulating polymers in α1-antitrypsin deficiency. Eur Respir J 2014;43:1501-1504.

[46] Gooptu B, Lomas DA. Conformational pathology of the serpins - themes, variations and therapeutic strategies. Annu Rev Biochem 2009;78:147-176.
[47] Davis RL, Shrimpton AE, Holohan PD, Bradshaw C, Feiglin D, Sonderegger P, et al. Familial dementia caused by polymerisation of mutant neuroserpin. Nature 1999;401:376-379.
[48] Davis RL, Shrimpton AE, Carrell RW, Lomas DA, Gerhard L, Baumann B, et al. Association between conformational mutations in neuroserpin and onset and severity of dementia. Lancet 2002;359:2242-2247.
[49] Coutelier M, Andries S, Ghariani S, Dan B, Duyckaerts C, van Rijckevorsel K, et al. Neuroserpin mutation causes electrical status epilepticus of slow-wave sleep. Neurology 2008;71:64-66.
[50] Hagen M, Murrell JR, Delisle MB, Andermann E, Andermann F, Guiot MC, et al. Encephalopathy with neuroserpin inclusion bodies presenting as progressive myoclonus epilepsy and associated with a novel mutation in the proteinase inhibitor 12 Gene. Brain Pathol 2011;21:575-582.
[51] Belorgey D, Crowther DC, Mahadeva R, Lomas DA. Mutant neuroserpin (Ser49Pro) that causes the familial dementia FENIB is a poor proteinase inhibitor and readily forms polymers *in vitro*. J Biol Chem 2002;277:17367-17373.
[52] Miranda E, Römisch K, Lomas DA. Mutants of neuroserpin that cause dementia accumulate as polymers within the endoplasmic reticulum. J Biol Chem 2004;279:28283-28291.
[53] Belorgey D, Sharp LK, Crowther DC, Onda M, Johansson J, Lomas DA. Neuroserpin Portland (Ser52Arg) is trapped as an inactive intermediate that rapidly forms polymers: implications for the epilepsy seen in the dementia FENIB. Eur J Biochem 2004;271:3360-3367.
[54] Miranda E, McLeod I, Davies MJ, Pérez J, Römisch K, Crowther DC, et al. The intracellular accumulation of polymeric neuroserpin explains the severity of the dementia FENIB. Hum Mol Genet 2008;17:1527-1539.
[55] Takehara S, Onda M, Zhang J, Nishiyama M, Yang X, Mikami B, et al. The 2.1-A crystal structure of native neuroserpin reveals unique structural elements that contribute to conformational instability. J Mol Biol 2009;388:11-20.
[56] Belorgey D, Hägglöf P, Onda M, Lomas DA. pH dependent stability of neuroserpin is mediated by Histidines 119 and 138; implications for the control of β-sheet A and polymerisation. Protein Sci 2010;19:220-228.
[57] Schipanski A, Lange S, Segref A, Gutschmidt A, Lomas DA, Miranda E, et al. A novel interaction between aging and ER overload in a protein conformational dementia. Genetics 2013;193:865-876.
[58] Gooptu B, Lomas DA. Polymers and inflammation: disease mechanisms of the serpinopathies. J Exp Med 2008;205:1529-1534.
[59] Gooptu B, Dickens JA, Lomas DA. The molecular and cellular pathology of α□-antitrypsin deficiency. Trends Mol Med 2014;20:116-127.
[60] Mayer NS, Stoller JK, Bartelson BB, Ruttenber AJ, Sandhaus RA, Newman LS. Occupational exposure risks in Individuals with PI*Z α_1-antitrypsin deficiency. Am J Respir Crit Care Med 2000;162:553-558.
[61] Stoller JK, Aboussouan LS. Alpha-1 antitrypsin deficiency. Lancet 2005;365:2225-2236.

[62] Dirksen A, Dijkman JH, Madsen F, Stoel B, Hutchison DCS, Ulrik CS, et al. A randomised clinical trial of α_1-antitrypsin augmentation therapy. Am J Resp Crit Care Med 1999;160:1468-1472.
[63] Dirksen A, Piitulainen E, Parr DG, Deng C, Wencker M, Shaker SB, et al. Exploring the role of CT densitometry: a randomised study of augmentation therapy in alpha1-antitrypsin deficiency. Eur Respir J 2009;33:1345-1353.
[64] Dickens JA, Lomas DA. Why has it been so difficult to prove the efficacy of alpha-1-antitrypsin replacement therapy? Insights from the study of disease pathogenesis. Drug Des Devel Ther 2011;5:391-405.
[65] Lange P, Celli B, Agustí A, Boje Jensen G, Divo M, Faner R, et al. Lung-Function Trajectories Leading to Chronic Obstructive Pulmonary Disease. N Engl J Med 2015;373:111-122.
[66] Chapman KR, Burdon JG, Piitulainen E, Sandhaus RA, Seersholm N, Stocks JM, et al. Intravenous augmentation treatment and lung density in severe α1-antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. Lancet 2015;386:360-368.
[67] Jedicke N, Struever N, Aggrawal N, Welte T, Manns MP, Malek NP, et al. α-1-antitrypsin inhibits acute liver failure in mice. Hepatology 2014;59:2299-2308.
[68] Mueller C, Flotte TR. Gene-based therapy for alpha-1 antitrypsin deficiency. COPD 2013;10:44-49.
[69] Massaro GD, Massaro D. Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats. Nature Med 1997;3:675-677.
[70] Stolk J, Stockley RA, Stoel BC, Cooper BG, Piitulainen E, Seersholm N, et al. Randomized controlled trial for emphysema with a selective agonist of the gamma type retinoic acid receptor. Eur Respir J 2012;40:306-312.
[71] Donahue JM, Cassivi SD. Lung volume reduction surgery for patients with alpha-1 antitrypsin deficiency emphysema. Thorac Surg Clin 2009;19:201-208.
[72] Hillerdal G, Mindus S. One- to four-year follow-up of endobronchial lung volume reduction in alpha-1-antitrypsin deficiency patients: a case series. Respiration 2014;88:320-328.
[73] Devlin GL, Parfrey H, Tew DJ, Lomas DA, Bottomley SP. Prevention of polymerization of M and Z α_1-antitrypsin (α_1-AT) with Trimethylamine N-Oxide. Implications for the treatment of a_1-AT deficiency. Am J Respir Cell Mol Biol 2001;24:727-732.
[74] Burrows JAJ, Willis LK, Perlmutter DH. Chemical chaperones mediate increased secretion of mutant α_1-antitrypsin (α_1-AT) Z: a potential pharmacologcial strategy for prevention of liver injury and emphysema. Proc Natl Acad Sci USA 2000;97:1796-1801.
[75] Teckman JH. Lack of effect of oral 4-phenylbutyrate on serum alpha-1-antitrypsin in patients with alpha-1-antitrypsin deficiency: a preliminary study. J Pediatr Gastroenterol Nutr 2004;39:34-37.
[76] Hidvegi T, Ewing M, Hale P, Dippold C, Beckett C, Kemp C, et al. An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. Science 2010;329:229-232.
[77] Kaushal S, Annamali M, Blomenkamp K, Rudnick D, Halloran D, Brunt EM, et al. Rapamycin reduces intrahepatic alpha-1-antitrypsin mutant Z protein polymers and liver injury in a mouse model. Exp Biol Med (Maywood) 2010;235:700-709.

[78] Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, et al. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature 2000;403:98-103.
[79] Pastore N, Ballabio A, Brunetti-Pierri N. Autophagy master regulator TFEB induces clearance of toxic SERPINA1/α-1-antitrypsin polymers. Autophagy 2013;9:1094-1096.
[80] Bouchecareilha M, Hutta DM, Szajnera P, Flotte TR, Balch WE. Histone Deacetylase inhibitor (HDACi) Suberoylanilide Hydroxamic Acid (SAHA) mediated correction of alpha-1 antitrypsin deficiency. J Biol Chem 2012;287 38265-38278.
[81] Wooddell CI, Blomenkamp KS, Kanner S, Chu Q, Hamilton HL, Wakefield DH, et al. A hepatocyte-targeted RNAi-based treatment for liver disease associated with alpha-1-antitrypsin deficiency. Program and abstracts of the 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD); November 7-11; 2014; Boston, Massachusetts; 2014.
[82] Sehgal A, Blomenkamp KS, Qian K, Simon A, Haslett P, Barros S, et al. Pre-Clinical Evaluation of ALN-AAT to Ameliorate Liver Disease Associated With Alpha-1-Antitrypsin Deficiency. Gastroenterology 2015;148:Supplement 1, pg S-975.
[83] Guo S, Booten SL, Aghajan M, Hung G, Zhao C, Blomenkamp K, et al. Antisense oligonucleotide treatment ameliorates alpha-1 antitrypsin-related liver disease in mice. J Clin Invest 2014;124:251-261.
[84] Lomas DA, Evans DL, Stone SR, Chang W-SW, Carrell RW. Effect of the Z mutation on the physical and inhibitory properties of α_1-antitrypsin. Biochemistry 1993;32:500-508.
[85] Skinner R, Chang W-SW, Jin L, Pei X, Huntington JA, Abrahams J-P, et al. Implications for function and therapy of a 2.9Å structure of binary-complexed antithrombin. J Mol Biol 1998;283:9-14.
[86] Mahadeva R, Dafforn TR, Carrell RW, Lomas DA. Six-mer peptide selectively anneals to a pathogenic serpin conformation and blocks polymerisation: implications for the prevention of Z α_1-antitrypsin related cirrhosis. J Biol Chem 2002;277:6771-6774.
[87] Parfrey H, Dafforn TR, Belorgey D, Lomas DA, Mahadeva R. Inhibiting polymerisation: new therapeutic strategies for Z α_1-antitrypsin related emphysema. Am J Respir Cell Mol Biol 2004;31:133-139.
[88] Zhou A, Stein PE, Huntington JA, Sivasothy P, Lomas DA, Carrell RW. How small peptides block and reverse serpin polymerization. J Mol Biol 2004;342:931-941.
[89] Elliott PR, Pei XY, Dafforn TR, Lomas DA. Topography of a 2.0Å structure of α_1-antitrypsin reveals targets for rational drug design to prevent conformational disease. Protein Science 2000;9:1274-1281.
[90] Parfrey H, Mahadeva R, Ravenhill NA, Zhou A, Dafforn TR, Foreman RC, et al. Targeting a surface cavity of α_1-antitrypsin to prevent conformational disease. J Biol Chem 2003;278:33060-33066.
[91] Mallya M, Phillips RL, Saldanha SA, Gooptu B, Leigh Brown SC, Termine DJ, et al. Small molecules block the polymerisation of Z α_1-antitrypsin and increase the clearance of intracellular aggregates. J Med Chem 2007;50:5357-5363.

[92] Tan L, Perez J, Mela M, Miranda E, Burling KA, Rouhani FN, et al. Characterising the association of latency with α1-antitrypsin polymerisation using a novel monoclonal antibody. Int J Biochem Cell Biol 2015;58:81-91.
[93] Irving JA, Miranda E, Haq I, Perez J, Kotov VR, Faull SV, et al. An antibody raised against a pathogenic serpin variant induces mutant-like behaviour in the wild-type protein. Biochem J 2015;468:99-108.
[94] Ordóñez A, Pérez J, Tan L, Dickens JA, Motamedi-Shad N, Irving JA, et al. A single-chain variable fragment intrabody prevents intracellular polymerisation of Z α1-antitrypsin. . FASEB J 2015;29:2667-2678.
[95] Rashid ST, Corbineau S, Hannan N, Marciniak SJ, Miranda E, Alexander G, et al. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J Clin Invest 2010;120:3127-3136.
[96] Wilson AA, Yin L, Liesa M, Segeritz CP, Mills JA, Shen SS, et al. Emergence of a stage-dependent human liver disease signature with directed differentiation of alpha-1 antitrypsin-deficient iPS cells. Stem Cell Reports 2015;4:873-885.
[97] Yusa K, Rashid ST, Strick-Marchand H, Varela I, Liu PQ, Paschon DE, et al. Targeted gene correction of α1-antitrypsin deficiency in induced pluripotent stem cells. Nature 2011;478:391-394.
[98] Patschull AO, Gooptu B, Ashford P, Daviter T, Nobeli I. In silico assessment of potential druggable pockets on the surface of α1-antitrypsin conformers. PLoS One 2012;7:e36612.

Figure legends

Figure 1. (A) Crystal structure of native α_1-antitrypsin, solved to atomic resolution (1.8 Å, PDB ID 3NE4, [98]) and shown in ribbon representation with key features indicated. The reactive loop (red) inserts as a central, 4th strand in β-sheet A (i.e. strand 4A) that is shown in blue, during stabilising conformational transitions, e.g. to execute antiprotease action or during polymerisation. Beta-sheet B is shown in gold, and β-sheet C in green, with strand names indicated. The arrow indicates the site of the Z (Glu342Lys) mutation, within the breach region where initial intramolecular loop insertion may occur. Other pathogenic mutations affecting α_1-antitrypsin and other members of the serpin superfamily also cluster within the shutter region that regulates patency of the reactive loop insertion site. Reactive loop insertion requires expansion of β-sheet A to close the lateral hydrophobic pocket, and so this can act as a site for allosteric control of conformational change. (B) Three models of α_1-antitrypsin polymerisation. Linkage motifs are indicated by boxes, intermolecular β-strand insertion/complementation sites by arrows. (i) Single strand (reactive loop) linkage model of polymerisation; (ii) β-hairpin (strands 5A+6A) linkage model; (iii) Triple-strand (C-terminal motif: β-strands 1C, 4B+5B) linkage model.

Figure 2. Z α_1-antitrypsin polymers have a 'beads on a string' appearance. Reproduced from [17] with permission.

Figure 3 Biology of α_1-antitrypsin (upper panel) and pathobiology of its misfolding, polymerisation and deficiency (lower panel) in the liver and the lung. The defined and proposed pathogenic mechanisms fall into 3 general categories:

(I) Toxic gain-of-function consequences of misfolding and polymerisation - intracellular

(II) Loss of physiological function due to lack of native α_1-antitrypsin

(III) Toxic gain-of-function of polymeric α_1-antitrypsin – extracellular

Images within diagram used with permission from reference [2].

Figure 4. Allosteric targeting of α_1-antitrypsin polymerisation by small molecules for drug development. Left panel: Purple mesh indicates initial volume within the lateral cavity for targeting of small molecules to block polymerisation [89, 91]. Right panel: Subsequent X-ray crystallographic studies further defined a minimal target within the pocket (cyan mesh upper panel, transparent volume, lower panel) against which smaller compounds could be targeted *in silico*, lower panel [13].

Fig 1A

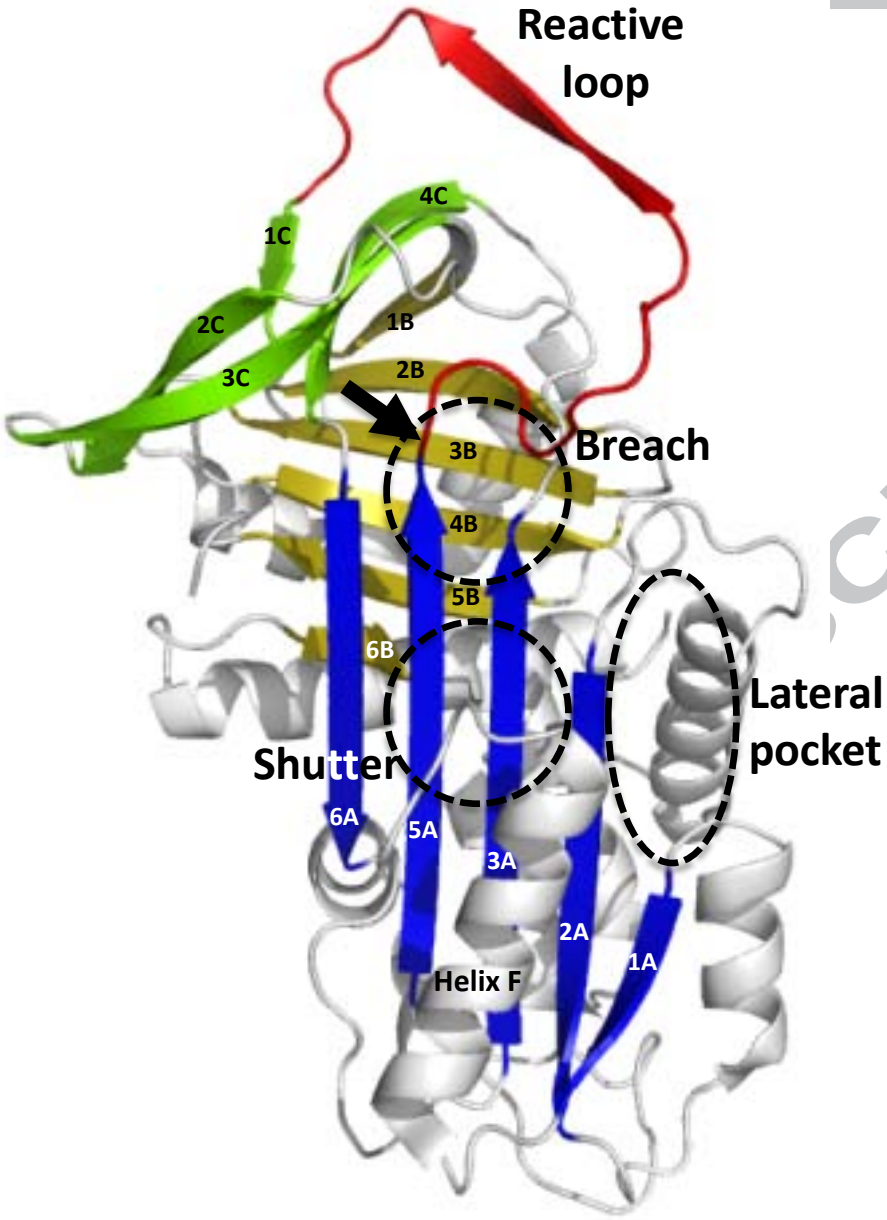


Fig 1B (i)

(i)

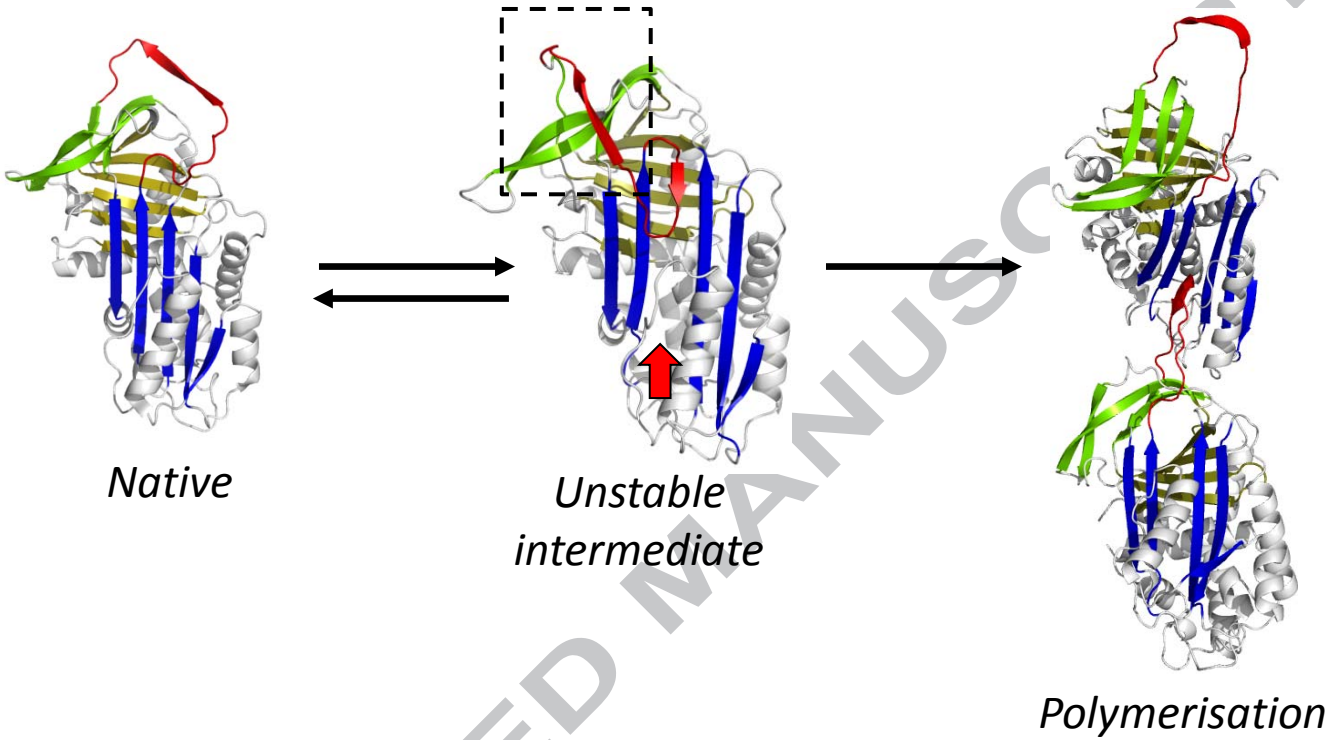


Fig 1B (ii) and (iii)

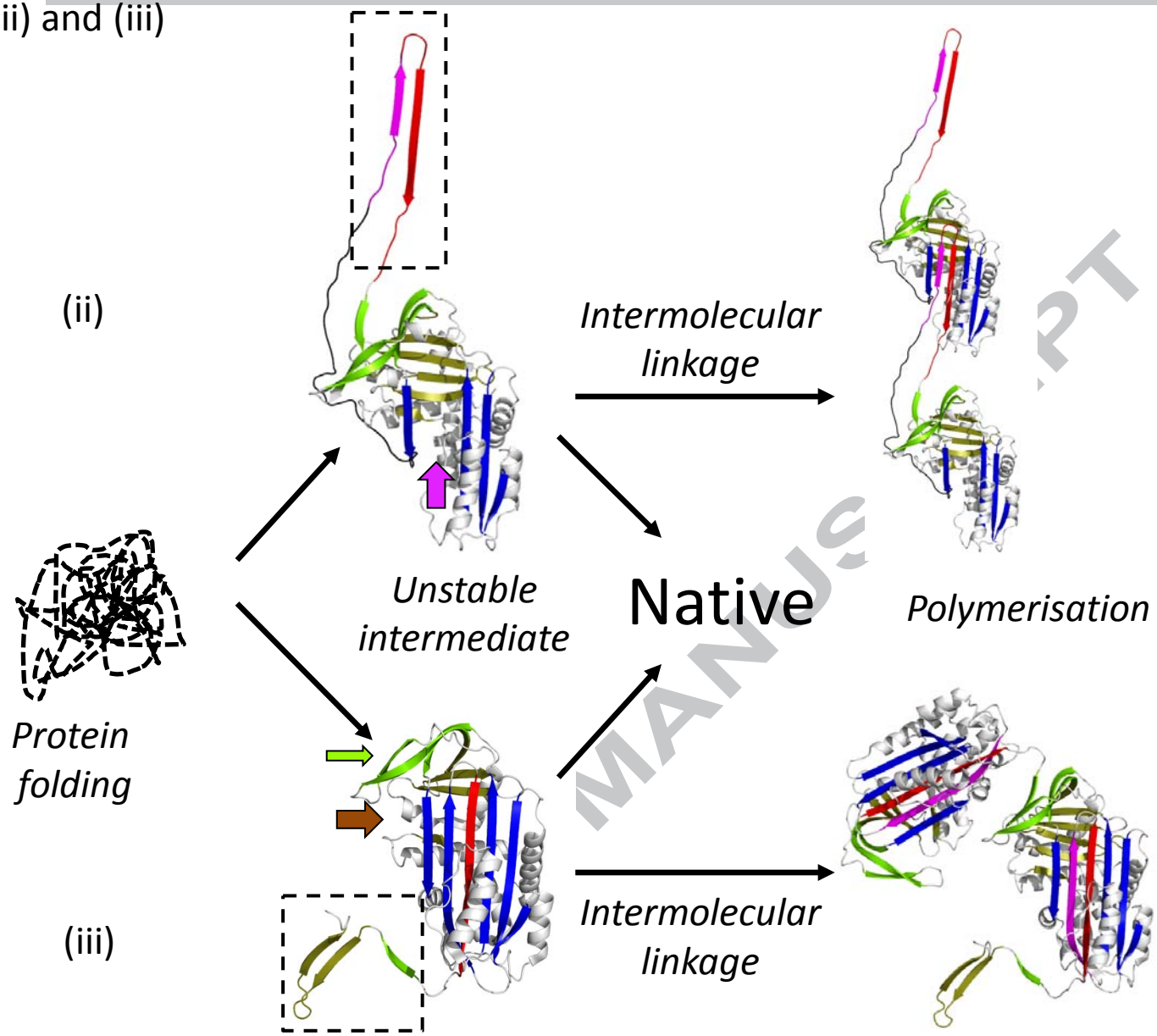


Fig 2

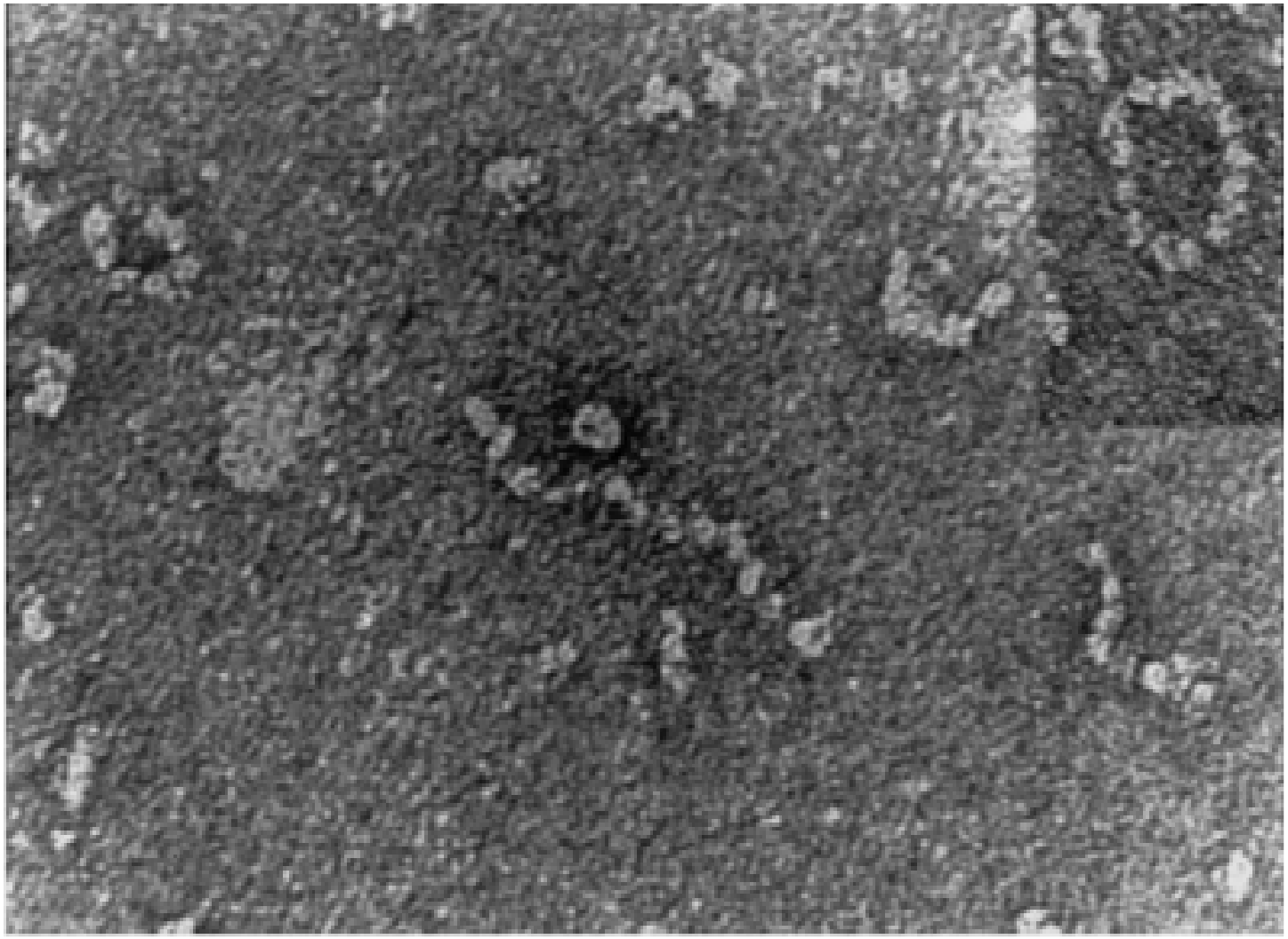
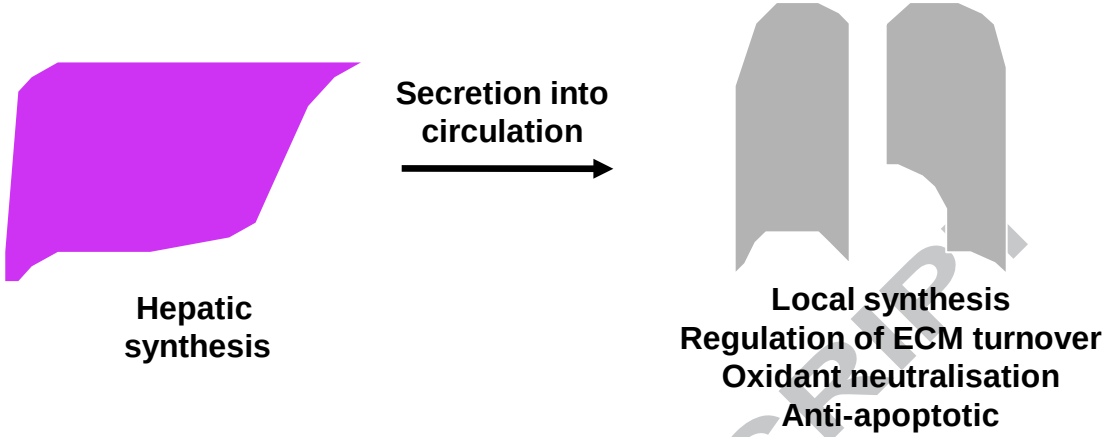


Fig 3

LIVER LUNG

Health



Disease

Ordered protein response (I)
- **Sensitivity to ER stress/UPR with "2nd hit" (I)**

Neonatal hepatitis (I)
Cirrhosis (I)
Hepatocellular carcinoma (I)

Excessive inflammation/emphysema:
Pulmonary antiprotease deficiency (II)
Interstitial • $_1$-antitrypsin polymers (III)
- **Proteolytic tissue damage (II)#**
- **Interstitial neutrophils, degranulation (III)#**
- **Apoptosis (II)#**
- **Antioxidant capacity (II)#**

Fig 4